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ENZYME SEPARATION AND PURIFICATION USING ELECTROFILTRATION

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Abstract
Membrane-based processes are playing critical role in the field of separation/purification of biotechnological products. Membranes became an integral part of biotechnology and improvements in membrane technology are now focused on high resolution of bioproduct. This manuscript provides an overview of recent developments and published literature in membrane technology, focusing on Electrofiltration.. that are now used for the separation and purification of enzymes. Electrofiltration present to overcome problems with fouling and concentration polarization in crossflow ultrafiltration of enzymes by using electrophoretic force. The result is an enhanced flux. The technique has many application. It has been successfully used on biomelecules [1], proteins [2] cleaning of waste water [3] inorganic metallic compounds [4] and water soluble polymers

Keyword : Electrofiltration, Electro-Ultrafiltration, Enzyme, proteins, Electric field

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1. Introduction

Enzyme are biomelecules that catalyze chemical reaction. In enzymatic reaction, the molecules at the beginning of the process are called substrates, and the enzyme converts them into different molecules, the products. Almost all processes in biological cell, enzymes need to occur at significant rates. Enzymes are known to catalyze about 4000 biochemical reaction. There are about more than 10,000 types of enzymes that have been found and more than 100 of them have been purified and crystal form. Enzyme molecular weight varied between 12,700 (ribonuclease) and 1000,000 (L-glutamate dehydrogenase, D-carboxylase).

Enzymes are proteins, protein conjugate or metalo protein. Enzymes are biological catalysts that very sensitive to temperature and pH for maximum activity. The enzyme easily inactivated by heat, chemicals, physical treatment, and easily hydrolyzed by other enzymes. Therefore the enzyme needs to get special treatment.

The fast growth in the field of biotechnology along with rapid commercialization of protein products has led to an increase in the demand for efficient, large-scale protein purification techniques. Techniques used in research laboratories (e.g. chromatography, electrophoresis, and affinity purification) are excellently suited for producing small quantities of protein. However, these processes are extremely difficult to scale-up and this factor restricts the scale of production. In addition to scale-up problems these techniques require complex instrumentation support to run efficiently, and give low throughput of product at an extremely high cost. Ultrafiltration (UF) processes are cost effective and can be fine-tuned to achieve high productivity and product purity at the same time. UF processes are also much easier to scale-up in comparison to chromatography and electrophoresis. In addition to this, UF modules are easy to clean and operate, and quite compact in design. Fouling of MF/UF membrane during practical application for protein separation resulted from its adsorption on membrane surface significantly increases hydraulic resistance to flow, which reduced filtration flux rate and induced unfavorable effect on efficiency and economics of protein recovery processes. Proteins are difficult foulants to deal because they readily adsorb onto membrane surface and pore walls. This leads to the formation of a secondary barrier that decreased permeate flux and changed solute selectivity. Fouling can occur by several forms in particular deposition of denaturated or agglomerated proteins at the surface of the membrane, or adsorption of proteins inside the pore structure of the membrane.

The significant flux decline with time due to membrane fouling is a potential limitation on the efficient use of ultrafiltration/microfiltration. Such flux decline is mainly attributable to the formation of highly resistant filter cake caused by accumulation of the enzyme solutes on the membrane surface. To overcome problems with fouling and concentration polarization in crossflow ultrafiltration (UF) of enzymes, various ingenious techniques have been developed for reducing the amount of cake forming, including crossflow filtration, dynamic filtration with rotating cylindrical membrane, upward and inclined filtration, and electrically enhanced filtration. In the present paper, the process design, theory and some applications of electrofiltration are presented.

2. Electrofiltration

Electrofiltration is a process combining a pressure gradient and an electrical potential gradient as driving force for separation. Electrofiltration has been investigated from the seventies but the procedure of electro-ultrafiltration as suggested by Bechold in 1925 has been further developed and improved over the past ten years.

According of electrofiltration definition, electrofiltration can be combination of microfiltration/ultrafiltration and electric field, microfiltration/ultrafiltration and electrodialysis, and electrodialysis which have porous membrane with pressure driven. A combination of ultrafiltration and electrodialysis.electric charge is known as electro-ultrafiltration. Electro-ultrafiltration is effective in decreasing the gel layer formation and in increasing filtration flux, owing to electrokinetic phenomena such as electrophoresis and electroosmosis.

Bergeman et.al designed a new filtration module that uses one ultrafiltration (UF) membrane and an electrical field as a driving force to perform the separation of peptides from a casein α_{S2} hydrolysate, with separation of electrolyte compartment. In replacement of ion-exchange membrane used in an electrodialysis (ED) module, Galier and Roux-de Balmann investigated the use of porous membranes. Young G. Park use use Electro-microfiltration to purify protein.Kappler and Posten reported the isolation of lysozyme from a mixture of lysozyme and bovine serum albumin (BSA) by two-sided electrofiltration using pressure and electric field gradients.

Other researchers reported incomplete isolation of single peptides from protein hydrolysates by electrofiltration with pressure and electric field gradients.

The study shows that membrane system produced with an electro-Microfiltration technique offers significant advantages.

2.1 Process Design

Two different configuration have been reported for electrofiltration. An electric field can be applied across the membrane with one electrode on either side of the membrane (figure 1a) or the electric field may be applied between the membrane and another electrode (figure 1b).

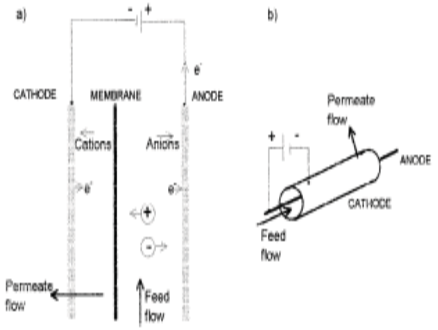

Figure 1. Schematic illustration of electrofiltration when (a) an electric field is applied across a flat sheet membrane and (b) a tubular membrane is used as an electrode

Usually, an electric field is applied across the membrane and is applied across micro- and ultrafiltration membranes in flat sheet, tubular and spirally wound modules.

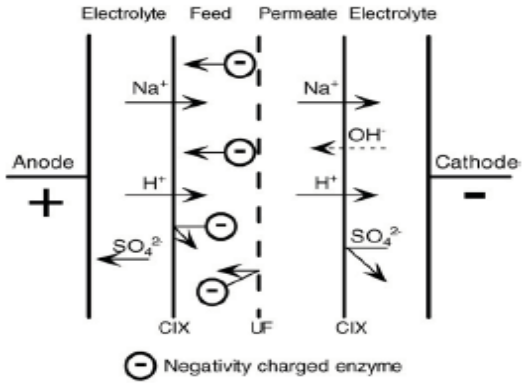

Figure 2.. Electro-ultrafiltration module

Wakeman and Tarleton calculated the trajectory of particles in crossflow microfiltration modules from an analysis of fluid velocity and electric field profiles. They compared plate, tubular and multi tubular modules. According to their results, tubular geometry leads to the most effective use of electrical power, when it is used as an aid to prevent membrane fouling.

Enevoldsen et al reported that the electrofiltration module is based on a commercial available electrodialysis module. To prevent direct contact between the enzymes and the electrodes, the crossflow EUF module is configured according to figure 2. The rig is shown in figure 3. The module consist of four chambers separated by an UF membranes surrounded by two cation exchange membranes. Flow spacers are used to enable the different streams. The channel height of electrolyte, feed and permeate chambers are 6.5 and 5 mm, respectively. It is possible to expand the module to contain several cells. It is necessary to shield the electrodes from the enzymes solution.

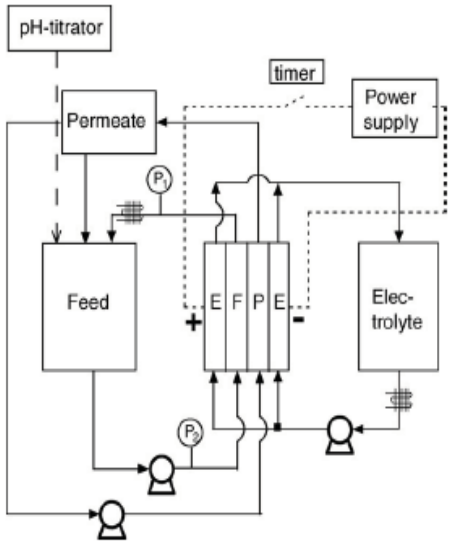

Figure 3. Electro-ultrafiltration set-up

Brisson et al designed microfiltration module (fig. 4) using consisted in two plates of poly vinyl chloride (PVC) in which platinized titanium electrodes have been cast. The feed channel was rectangular and its cross-section dimensions are 0.095 m x 0.0018 m. The separation distance between electrodes was 0.003 m and the effective electrode and membrane areas were, respectively, 0.0139 and 0.0159 m^2.

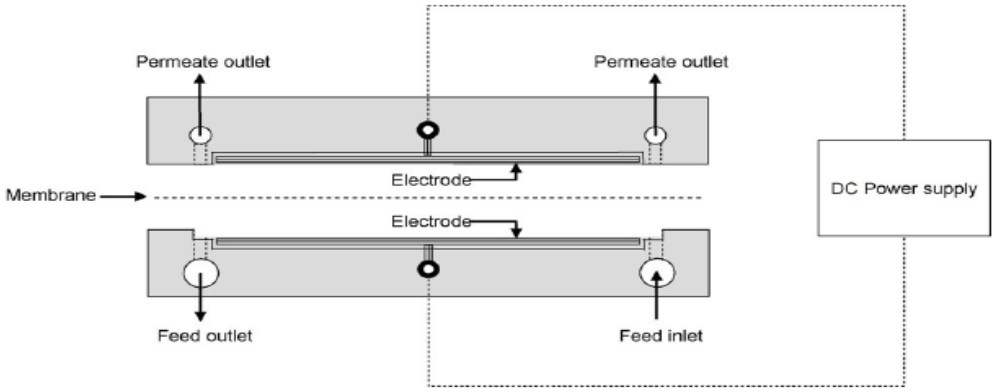

Figure 4. Schematic representation of the EMF module

2.2 Materials membrane and membrane support

Table 1. State of The Art of Material Membrane Electrofiltration

year	Pore size	Materials membrane Electrofiltration	researchers
2009	10 kDa	ZrO_2 mineral membranes	Firdaous *et al*
2008	50 kDa	Polyether sulfone (PES)	B.Sarkar, *et al*
2007	0.5 µm	Polyvinylidine fluoride (PVDF)	Brisson *et al*
2007	10 kDa	PVDF ETNA + Cation Exchange membrane used RELAX-CMH membrane	Enevoldsen *et al*
2007	95 kDa	In organic composites membranes (TiO_2/Al_2O_3)	Gordon *et al*
2006	20 kDa	Cellulose ester UF membranes + Neosepta CMX-S cationic and Neosepta AMX –SB anionic membrane	J.F. Poulin, *et al*
2006	0.1 µm	PES	Hofmann,R. *et al*
2005	0.22-3.5µm	PVDF	Young G.Park
2000	30 kDa	Asymetric polysulfone	E.Iritani *et al*
1999	50 kDa	Polysulfone flat sheet membrane	N.Mameri *et al*
1998	50 kDa	Polyether sulfone (PES)	Zumbusch *et al*
1998	50 kDa	Polyamide (PA)	Zumbusch *et al*
1998	30 kDa	Regenerated cellulose	Zumbusch *et al*
1998	20 kDa	Polyvinylidine fluoride (PVDF)	Zumbusch *et al*
1998	0.2 µm	Polyamide	Tom Weigert *et al*
1998	0.2 -8 µm	Cellulose nitrate	Wakeman, R.J
1996	20 kDa	Ceramic membrane (Cintered carbon pipe with an inner layer of ZrO_2)	M.Hakoda, *et al*
1993	100 kDa	Asymetric polysufone	S. Lentach *et al*

The membrane material can either be electrically conductive or non conductive. Another possibility in electrofiltration, when the membrane is made of metal. Carbon or an other conductive material, is to use the membrane as an electrode.

The membrane support may form one of the electrodes, usually the cathode, and is often made of stainless steel. The anode is on the feed side. According to Bowen, one of the best anode materials is titanium, coated with a thin layer of nobel metal such as platinum. According to Jagannadh and Muralidhara, one of the major restrictions in the commercial implementation of electric-field enhanced technologies is the lack of suitable corrosion-resistant and inexpensive electrode materials. The design and location of the electrodes are important for uniform distribution of the electric field strength along the membrane and for reducing energy consumption during filtration.

2.3 Performance

Two electrodes are positioned parallel to the membrane. An Electric field is generated by applying a voltage to the electrodes. The field vector is perpendicular to the membrane particles that carry a net charge are moved towards the electrode with the opposite sign. This phenomenon is called electrophoresis. At the same time the electric field generates electroosmosis in the membrane. If the membrane material carries a net charge, the electric field generates a water flux through the pores of the membrane. Both phenomena increase the permeating flux, if the membrane and the particles are negatively charged.

2.3.1 The influence of Electric field strength

Enevoldsen *et al.,* (2007) reported flux improved 3-7 times for enzymes with a significant surface charge at an electric field strength of 1600 V/m compared to conventional Ultrafiltration . An important parameter in the design and modeling of an electrofiltration process is the electric field strength. Huotari H.M. *et al.,* (1999) investigated in a flat sheet system the electric field strength, E, is easily calculated from its definition:

$$\mathrm{E} = \frac{\phi}{L} \qquad (1)$$

Where ϕ is the electric potential (voltage) and L is the distance between electrodes. However, in a tubular system equation (1) must be modified. Wakeman *et a.,*. [1987] studied the electric field strength distributions between two concentric cylinder can be calculated according to equation (2) :

$$\mathrm{E} = \frac{\left(\Phi_o - \Phi_i\right)}{r \log(r_0 / r_i)} \qquad (2)$$

Where Φ_o is the electric potential at the outer electrode, Φ_i is the electric potential at the inner electrode, r is the radial coordinate, r_o is the radius of the outer electrode and r_i is the radius of the inner electrode. According to some authors, electric field strength may not be calculated from the overall applied voltage, since the drop in voltage at the electrode-solution interfaces (overpotential) is unknown. Hence, E is calculated according to Ohm's law from the unambiguous values of the current (*I*), the cell dimensions, and the known conductivity of the bulk solution (λ_o):

$$\mathrm{E} = \frac{I}{\lambda_o} \qquad (3)$$

E.Iritani *et al.,* (2000) reported that the electric field strength *E* can be calculated from

$$E = \frac{i}{k\ A} \qquad (4)$$

Where i is the electric current, *k* is the specific electric conductivity, and A is the effective membrane area.

H.Yukawa *et al*.,(1983) invstigated flux was proportional to E, where

$J_E = \mathrm{k\ E} = 2{,}35 \times 10^{-9} \exp(0{,}31/C^*)\ \mathrm{Re}^n\ \mathrm{E}$ (5)
for $3000 < \mathrm{Re} < 5 \times 10^4$

$J_E = \mathrm{k\ E} = (3.7 \times 10^{-8} - 1.77 \times 10^{-7}\ C^*)\ \mathrm{Re}^{-0.05}\ \mathrm{E}$ (6)
for $500 < \mathrm{Re} < 2200$

According to Eq. (1,2), J_E is proportional to E when C_b ia constant in the steady state. The relation between electric power P and electric field strength E is expressed by

$$\mathrm{E} = \left(\frac{\mathrm{P}}{\mathrm{ALk}}\right)^{0.5} \qquad (7)$$

Based on Ohm's law, where A is logarithmic mean area of electrodes. Consequently, J_E is proportional to $P^{0.5}$ theoretically.

The following effects have to be taken into consideration when an electric field is applied: electrophoresis, electroosmosis and electrochemical reactions.

2.3.1.1 Electrophoresis

An electric field affects the trajectories of charged particles and colloids and can thus prevent them from being deposited on the membrane. This is called electrophoresis. So the electrophoresis is the migration of charged particles when an electric field is applied, and it is described by the electrophoretic mobility. The electrophoretic mobility μ is the

migration velocity (v_{ep}) of the charged particle in an electric field with the electric field strength E.

$$\mu = \frac{v_{ep}}{E} \tag{8}$$

In crossflow electrofiltration the particle surface charge is used to distract the particle flow towards the membrane and consequently to prevent particle deposition at the membrane by the application of an oppositely directed electric field.

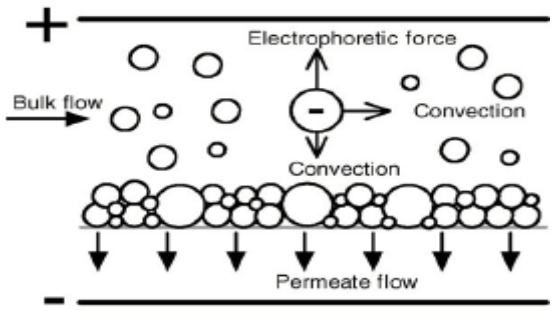

Figure 5. Principle of electrofiltration

The electric field causes an additional electrophoretic force F_{ep}

$$F_{ep} = 3\pi d\eta\lambda\mu E \tag{9}$$

2.3.1.2 Electroosmosis

If the membrane has charges pore walls, there will be an excess of counter ions within the pore. These counter-ions will move in an applied electric field and drag the solvent (water) with them. The resulting water flux is called the electro-osmotic flow or electroosmosis. Electroosmosis is usually neglected in microfiltration where the permeate flow through the membrane is determined by hydrodynamic effects.

2.3.1.3 Electrochemical reactions

Apart from electrophoresis and electro-osmosis, other effects occur in electrofiltration, such as electrochemical reaction. An electrochemical reaction is a chemical process involving the transfer of charge to or from an electrode. A typical cathodic process in aqueous systems without noble metal ions is the formation of hydrogen gas:

$2H_2O + 2e^- \rightarrow H_2(g) + 2\ OH^-$ ($E_0 =$ -0.83 V, leading to a pH increase)

(10)

$2\ H_3O^+ + 2e^- \rightarrow 2H_2O + H_2(g)$ ($E_0 = 0$ V) (11)

$M^{n+} + ne^- \rightarrow M$ (12)

Conversely, at the anode, an otherwise stable substance is oxidized by the removal of electrons from the substances to the electrode. A relevant example could be:

$2H_2O \rightarrow O_2\ (g) + 4H^+ + 4e^-$ (anode, +0.40V, leading to a pH decrease)

(13)

$M \rightarrow M^{n+} + ne^-$ (14)

M represents the electrode material. In case of inert anode materials such as platinum, the last reaction can be depressed. Electrode materials that are not fully inert, such as stainless steel, will however undergo some oxidation during electrofiltration, giving rise to multivalent metal ion such as Fe^{3+}. The main reaction are nevertheless the production of pH determining protons and hydroxide ions.

A high current density indicates a high degree of simultaneous oxidation and reduction. If the conductivity of the liquid increases, the current density increases causing electrochemical reactions to take place at the electrodes. According to some authors, the effective operation of electrofiltration is limited to the same conductivity range as for electro-osmotic dewatering, which is 0.10 to mS cm^{-1} .

Mameri *et al.*, [1999] observed the dissociation of the water and exhaust gas in each electrode compartment. Oxidation of Hydroxyl ions onto the deployed metal sheet, representating the anode, led to the formation of microscopic oxygent bubbles.

$H_2O + OH^- \rightarrow ½\ O_2 + H_3O^+ + e^-$ (15)

The production of hydronium ions explains the decrease in pH of buffered solutions during the electrofiltration. Enhancement of the permeate flux may be attributed to the microscopic bubbles produced by the anode near the membrane, which considerably reduced the polarization layer resistance.

2.3.2 The influence of pH and ionic strength

α-amilase enzymes contain a group of amino acids that containing carboxylic ($-COO^-$) and ammonium ($-NH_3^+$) groups. The simultaneous presences of both groups cause the enzyme to act as an amfother, able to react with acids and base simultaneously and producing cation and anion. This can cause the pH of the solution to determine the electric capacity of the enzyme. The value of pH where an enzyme does not have such capacity is called isoelectric point (pI). An enzyme will have a negative charge at a pH above the pI and have a positive charge below the pI. The difference in the enzyme's carriage will influence the mobility values as well as the diffusion coefficient. Taking into account that these two parameters determine the behaviour of the mass transfer, hence, the loading difference can be utilized to separate the enzyme based on electrical interactions between enzyme-membrane, enzyme-enzyme or enzyme-solvent.

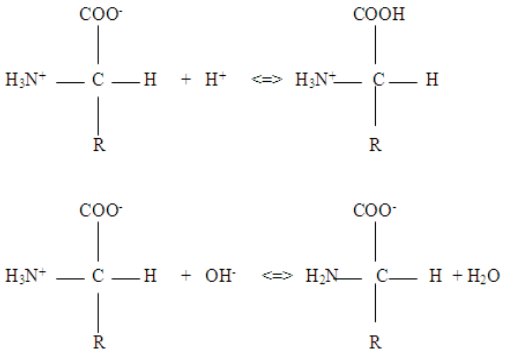

Figure 6. Amphoter nature of the protein

Studies show that membrane filtration for protein and enzymes are highly influenced by the natural characteristics and interactions between solutes (physicochemical interactions). The interaction between membrane and solute can occur in a form of electricity loading, hydrophobic or even loading transfer. The advantage is that the transmission from the solute via the membrane can be manipulated through the concentration of α-amilase enzyme. Physicochemical interactions such as pH and ionic strength or other parameters such as transmembrane pressure configuration system (Zulkali, Ahmad and Derek, 2004).

3. Electrofiltration model

There exists no general model to predict the influence of an electric field in crossflow membrane filtration. Different authors have developed different models. Most of the models are modified expressions of the resistance-in series model and the gel layer.
Yukawa *et al.,* (1983) based his model on the views of Jorden and Moulik, who first postulated an influence on dewatering not only by electroosmosis, but also by electrophoresis. Based on the equation of cake building filtration, Yukawa developed an equation for pressure electrofiltration. Electroosmosis is the moving of fluid in an electric field, and therefore Yukawa considered electroosmosis as an additional pressure added to the applied hydraulic pressure. Electrophoresis reduces the filtration velocity of the particles. If the electric field reaches a critical strength, the electrophoretical velocity equalizes the viltration velocity of the particle. If the electric field reaches a critical strength, the electrophoretical velocity equalizes the filtration velocity, and the particle float. The resulting velocity is zero, and there is no cake build up. Yukawa considered this effect using an electrophoretic coefficient $(E_{cr} - E)/E_{cr}$. This leads to a principal equation of pressure electrofiltration.

$$\frac{dV_L}{dt} = \frac{(\Delta p_H + \Delta p_E).A}{\eta.\left(r_c.K.\left(\frac{E_{cr}-E}{E_{cr}}\right).\frac{V_L}{A} + R_m\right)} \qquad (16)$$

V_L is the filtrate volume, t the time, ΔP_H the applied hydraulic pressure, ΔP_E the electroosmotic pressure, A the filtration area, η the dynamic viscosity, r_c the specific cake resistance, K is a concentration factor composed of the porosity of the bulk ε and the volume concentration of the suspension c_v:

$$K = \frac{c_V}{1\text{-}c_V - \varepsilon} \qquad (17)$$

E_{cr} is the critical electric field strength, E is the electric field strength and R_m is the resistance of filter medium.

4. Aplication, Limitation and Development of Electrofiltration

Electrofiltration has been successfully used on biomolecules, proteins, cleaning of waste water and water soluble polymers. Yukawa *et al.,* (1983) used

electro-ultrafiltration to separate and thicken colloidal solutions. Dou *et al.,* (2000) reported the electro-ultrafiltration (EUF) technique was used to measure the concentrations of total EUF-extractable Nitrogen (EUF-N_t), ammonium-N (EUF-NH_4^+-N) and nitrate-N (EUF-NOI_3^-N). [44]

Electrofiltration can be effective means of reducing both the concentration polarization and membrane deposition but it has some drawbacks which make this methods uneconomical and difficult to handle for certain processes. The disadvantages of this process are : (a) limitation of the process stream for relatively low conductivity of feed stream, (b) a high-energy requirement, (c) substantial heat production, and (d) changes in the process feed due to reaction at the electrode. For this reason, attention has been directed to the use of pulsed electric fields.

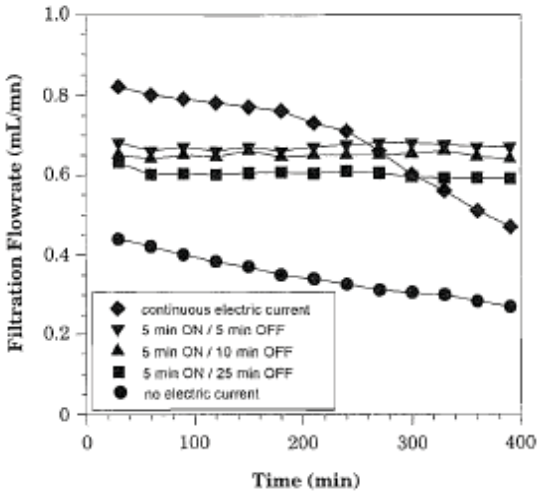

Figure 7. Averaged filtration flowrates under pulsed electric current with different ON/OFF duration ratios, Initial BSA concentration C_o =0.5 g/L; pH =8; ΔP =1.2 bar; electric current I=10 mA; crossflow rate, 50 mL/min

A pulsed electric field consumes less energy than a constant field. This process has the same mechanism at work in preventing fouling as conventional electrofiltration. The only differences is that in pulsed electric fields, the electric field can be applied at certain intervals, which can be adjusted to suit the process. In some cases, this process can enhance the flux better than the conventional electrofiltration.

Bowen and Sabuni [1992] utilized the pulsed electric field as a tool for cleaning microfiltration membrane fouled by titanium dioxide colloid. Kim and Lee [1997] examined the app;icability of the pulsed electric field in a crossflow ultrafiltration for the concentration of bovine serum albumin.

The other development of electrofiltration can be combine with turbulence promoter and ultrasonic fields.

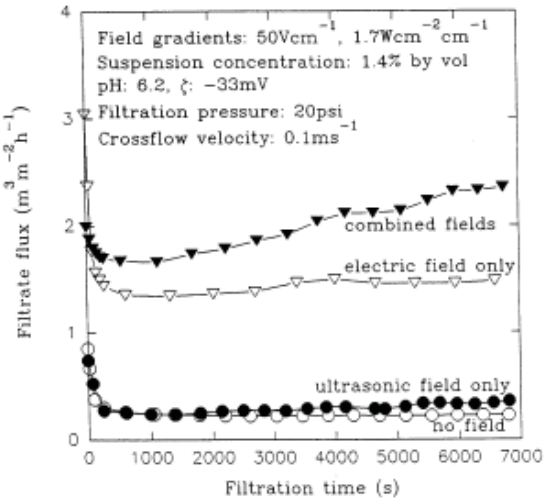

Figure 8. Synergy between electric and ultrasonic fields during the filtration of China clay suspensions

Conclusions

Ultrafiltration and microfiltration is being widely used to separate and purify enzyme and to remove small molecular solutes. The efficiency of UF/MF is limited by concentration polarization and fouling, One of the promising ways of controlling the concentration polarization and fouling is using electrofiltration. Although the application of electrofiltration is very effective in reducing concentration polarization and fouling, it has disadvantages including a high energy requirement, temperature change due to the joule heating, chemistry change of the process solution due to electrode reaction and electrodeposition of protein molecules on electrode surface. The development electrofiltration must be consider to overcome its problem. The one way is used pulsed electric field in the electrofiltration.

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